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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/771,949	02/03/2004	Paul A. DiTullio	21578-002 CON	3766
30623 7590 12/26/2007 MINTZ, LEVIN, COHN, FERRIS, GLOVSKY AND POPEO, P.C. ONE FINANCIAL CENTER BOSTON, MA 02111				
			EXAMINER SCHNIZER, RICHARD A	
			ART UNIT 1635	PAPER NUMBER
			MAIL DATE 12/26/2007	DELIVERY MODE PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No. 10/771,949	Applicant(s) DITULLIO ET AL.	
	Examiner Richard Schnizer, Ph. D.	Art Unit 1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

- 1) ☒ Responsive to communication(s) filed on 29 October 1976.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

- 4) ☒ Claim(s) 1, 5, 6, 8, 11, 14, 18, 21 and 24-27 is/are pending in the application.
- 4a) Of the above claim(s) _____ is/are withdrawn from consideration.
- 5) ☐ Claim(s) _____ is/are allowed.
- 6) ☒ Claim(s) 1, 5, 6, 8, 11, 14, 18, 21, 24-27 is/are rejected.
- 7) ☐ Claim(s) _____ is/are objected to.
- 8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on _____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some * c) ☐ None of:
1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

- | | |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892) | 4) <input type="checkbox"/> Interview Summary (PTO-413)
Paper No(s)/Mail Date. _____ |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948) | 5) <input type="checkbox"/> Notice of Informal Patent Application |
| 3) <input type="checkbox"/> Information Disclosure Statement(s) (PTO/SB/08)
Paper No(s)/Mail Date _____ | 6) <input type="checkbox"/> Other: _____ |

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 10/29/07 has been entered.

Claims 2, 8, 12, 13, 17, 2, 23, 25, and 26 have been canceled.

Claim 27 was added.

Claims 1, 5, 6, 8, 11, 14, 18, 21, 24-27 are pending and are under consideration in this Office Action.

After further consideration and review of the prior art, the enablement rejection of claims 1, 5, 6, 7, 14, 21, and 24 is withdrawn. These claims are drawn to methods of delivering DNA to a chicken spermatogonial cell in vivo, and were previously rejected as lacking enablement because the only purpose set forth in the specification for performing the claimed methods was the production of transgenic birds. This purpose is not considered enabled for the reasons set forth in the enablement rejection of claims 18 and 27, below. However, the prior art taught that DNA delivery to mouse spermatogonial cells in vivo could be used to study various facets of development (see e.g. Yamazaki et al (Biol. Reprod. 59: 1439-1444, 12/1998) at abstract, page 1439, column 2, second full paragraph, and especially page 1442, column 2, first full

Art Unit: 1635

paragraph. So it is considered to have been readily apparent to one of ordinary skill in the art prior to the time of the invention that one could use DNA delivery to spermatogonial cells for a purpose other than making transgenic animals. Note that Yamazaki was not applicable as prior art in the parent application (USSN 09/247,246, now US 6,686,199), because that application enjoyed priority to US Provisional application 60/073,386, filed 2/9/1998. The effective filing date of the instant application is 2/9/1999 because 60/073,386 does not disclose chickens.

Claim Interpretation

Claim 27 is directed to a method of producing transgenic sperm by infusing a transgene into a spermatogonium of a prepubertal chicken testicle in vivo and applying an electrical current to said testicle to increase uptake of said transgene, wherein semen collected from said testicle comprises said transgene. The term "transgenic" is generally interpreted in the prior art as meaning incorporated into the genome. That is, a "transgenic sperm" would generally be considered to be one in which a transgene has been inserted into a chromosome of the sperm cell. However, Applicant is entitled to be their own lexicographer, and the specification at page 4 states that a transgene need not be an integral part of a chromosome. Accordingly, "transgenic sperm" is interpreted as embracing sperm comprising a foreign gene that has not necessarily been integrated into a chromosome. On the other hand, the term "transgenic animal" is recognized in the art as referring to an animal that has a stable, heritable copy of a transgene that is not lost during development. See for example, Cameron (Mol. Biotech. 7:253265,

Art Unit: 1635

1997) who indicates that chromosomal integration is an inherent part of each of the common methods of transgenic animal production. See the paragraph bridging pages 254 and 256,, the first sentence of section 3.2 on page 255, and first sentence of first full paragraph on page 256. See also Wheeler et al (Theriogenology56:1345-1369, 2001) who stated that the "objective of transgenic technology is to produce animals that have stable incorporation of foreign DNA in their germ line." The term "transgenic animal" is term is not otherwise defined in the specification, and it will be interpreted according to its art-recognized meaning, i.e. requiring a stable, heritable copy of he transgene.

Priority

Applicant's amendment filed 10/29/07 perfected the priority claim to 09/247,246 (now US 6,686,199).

Claim Objections

Applicant's amendments overcame the previous objections.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 18 stands rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claim 18 is directed to a method of making a transgenic chicken by delivering a DNA to a testicle of a chicken, harvesting sperm cells from the chicken, and contacting an ovum with said sperm cells under conditions suitable for fertilization.

The specification teaches that transgenic animals in general are used for xenotransplantation, pharmaceutical production, protein production, and the study of human diseases. The specification taught no specific use for transgenic chickens. The prior art indicated interest in the use of transgenic chickens as bioreactors for protein production. The prior art of record did not envision the use of chickens for xenotransplantation or the study of human diseases.

Claims 18 is not considered to be enabled due to the high level of unpredictability of making transgenic birds as evidenced by the state of the art at the time of the invention, and due to the lack of adequate guidance and working examples in the specification. In particular, and pertinent to claim 27, the specification as filed did not teach how to predictably obtain integration of transgenes into the chromosomes of spermatogonia or sperm, or into the genomes of offspring of treated birds. Accordingly the specification did not teach how to make a true transgenic bird, i.e. one that comprises a transgene physically integrated into a chromosome.

Afanassieff et al (Avian Dis. 40 :841-852, 1996) taught intratesticular inoculation of avian leukosis retrovirus into adult and 1-week old brown leghorn chickens to investigate the possibility of producing transgenic chickens. However, no evidence of viral nucleic acid was detected after 6 weeks in prepubertal birds, and no viral nucleic acid was present in the semen of the adult birds (see page 845, column 2, first full paragraph, and paragraph bridging columns 1 and 2 on page 846). The data are consistent with elimination of virally-infected cells by the host immune system. See abstract, and first full paragraph of column 2 on page 849.

Li et al (Transg. Res. 4 :26-29, 1995) taught delivery of transgenes to primordial germ cells of the germinal crescent by gene gun. Chimeric hatchlings raised to maturity contained transgenes in their sperm. These birds were mated to produce G1 offspring. Twenty percent of the G1 offspring retained the transgene, but in the majority of cases, the DNA disappeared by maturity. See abstract. Li did not assess gene expression.

Ebara et al (Asian J. Androl. 1(3) : 139-144, 1999) taught transfection with microinjected naked DNA of germinal crescent cells in male and female chickens to produce chimeric birds. DNA was subsequently detected in the sperm cells of male chimeric chickens (see page 141, column 2, second and third full paragraphs and Table 4). Chimeric birds were bred, and transgene inheritance and expression in offspring was assessed. In no case did expression persist past the late embryo stage, and the introduced DNA was no longer detectable after 4 months in any bird. See item 3.2, Fig. 2, and Table 2 on page 141; paragraph bridging pages 142 and 143.

Sugihara et al (Comp. Biochem. Phys. B 125:47-52, 2000) taught foreign gene expression in quail testes by in vivo electroporation. Vectors used included episomally replicating vectors, and non-replicative vectors that would require chromosomal integration for stable transfection (see Fig. 1 on page 49). Regardless of the type of plasmid used, gene expression in testis was transient, even though a variety of constitutive promoters was used (CMV promoter, SV40 promoter/enhancer, RSV LTR, and beta actin promoter/enhancer, see Fig. 1).

In summary, at the time the invention was filed, no bird expressing a transgene had been produced by methods in which nucleic acids were delivered to progenitors of sperm cells, regardless of the age of the bird at the time the transgene was introduced. As a result the field of making transgenic birds by genetic modification of spermatogonia is considered to be immature, and highly unpredictable.

The instant specification provides no working example. Guidance in the specification as to how to improve on the results in the prior art is limited to the suggestion of the use of selectable markers and corresponding drugs to select for spermatogonial cells comprising the transgene. However, there is no precedent in the prior art of record for such in vivo selection of spermatogonial cells. Also, it is clear that such selection would, if it were feasible, only select for the presence of the transgene, and not necessarily for transgene integration. As seen in the art discussed above, non-viral transgenes that were present in spermatogonia did not become stably integrated into the genome, were not expressed in transgenic hatchlings, and were subsequently lost.

In view of the immature state of the art as discussed above, the high level of unpredictability, and absence of any working example in the specification, and the lack of adequate guidance, one of skill in the art could not practice the invention as intended (i.e. for the production of transgenic chickens or transgenic sperm) without undue experimentation.

Response to Arguments

Applicant's arguments filed 10/29/07 have been fully considered but are unpersuasive.

Applicant asserts that the invention is directed to gene transfer and that problems associated with the production of transgenic chickens in the prior art are overcome by targeting the spermatogonia of male testicles at a time to facilitate gene integration.

Applicant addresses each of the references relied upon by the Examiner for establishing the unpredictable state of the art.

Regarding the viral delivery technique of Afanassieff, Applicant indicates that the host immune system is specifically designed to mount a response to infection by foreign bodies so elimination of the virus from the testes is to be expected. Applicant also indicates that one would not expect retroviral infection to work well, one would instead expect the virus to integrate and become dormant. It is unclear how the method of claim 18 overcomes these disadvantages. The method of claim 18 does not limit the means of gene delivery, i.e. does not exclude viral delivery, and the specification specifically states that viral delivery may be used (see page 3, lines 15-17). The claim

recites no requirement for electroporation. Further, Applicant does not address the case in which the delivered transgene encodes a protein not native to the chicken, which if expressed in the spermatogonial cells would also be subject to an immune response. The claims are not limited to DNA encoding a self antigen. Accordingly Applicant's arguments regarding Afanassieff are unpersuasive.

Li and Ebara, taught gene transfer to germinal crescent cells and consequent unstable gene transfer to offspring, but not the production of transgenic animals comprising a heritable copy of the transgene. Applicant asserts that these results validate and support the enablement of the claimed invention because they demonstrate the feasibility of producing transgenic chickens. This is unpersuasive because no transgenic chickens were produced. Instead the foreign DNA disappeared from the animals, and no expression was detected past the embryonic stage of development. The specification does not teach how to use an animal comprising a transgene that is not expressed or is lost and not inherited.

Applicant indicates that the loss of the transgene can most likely be attributed to the choice of primordial germ cells (germinal crescent cells) as target cells because these cells divide slowly, and therefore make integration unlikely. This is unpersuasive for two reasons. First, Applicant's speculation is not supported by evidence and does not carry the weight of evidence. Second, the nucleic acids of Li and Ebara ended up in some cases in offspring. Applicant has not explained how this is possible if the cells containing the nucleic acids did not differentiate into spermatogonia, and then into sperm. If the nucleic acids were located in spermatogonia and spermatids, then the

Art Unit: 1635

basis of Applicant's arguments regarding the choice of primordial germ cells is unclear.

If some step that allows stable delivery and inheritance of the transgene occurs when the DNA is in spermatogonial of sperm cells, then why didn't it happen in the methods of Ebara and Li, wherein the DNA evidently passed through these cells on the way to the offspring?

Applicant refers in the paragraph bridging pages 6 and 7 of the response to the Declaration of Paul A. DiTullio, filed 10/5/06 for evidence that DNA was stably integrated into the chromosomes of chicken sperm using the instantly claimed methods. Applicant states that integration was proven "since an integrated transgene appears as a distinct fluorescent spot, as opposed to the dispersed fluorescence of unintegrated DNA." This statement finds no support in the Declaration of 10/5/06, which simply indicates results as "positive" or "negative" for fluorescence and does not characterize the nature of the fluorescence as distinct or dispersed. Accordingly the Declaration is not persuasive in this regard.

Finally, Applicant indicates that the Sugihara reference is not relevant to the presently claimed invention because it deals strictly with expression of a transfected gene in chicken testis rather than with production of transgenic sperm. This is unpersuasive because the specification as filed does not teach how to use a transgenic animal that does not express the transgene, and there is no evidence of record to indicate that transgenes that are silenced in sperm development will be reactivated in any tissue in progeny animals, particularly in a predictable manner that will provide some use. With regard to persistence of the transgene, Applicant relies on the results

of Ebara for evidence that the transgene can be transmitted to progeny. Applicant concludes that because a foreign gene was detected in the offspring of Ebara, the offspring were still transgenic. This is unpersuasive because the totality of the evidence indicates that none of the DNA of Sugihara or Ebara integrated into a chromosome of a spermatogonial cell, sperm cell, or of any cell of any offspring. In other words, the offspring were not transgenic. Instead, the totality of the evidence of record indicates that transfection of spermatogonia or primordial germ cells does not lead to detectable integration nor the recovery of transgenic animals with stably integrated, heritable foreign DNA. Furthermore, as stated above, the specification as filed did not teach how to use any transgenic animal that fails to express the transgene, and in any case, the claim is not limited to such animals.

For these reasons the rejection is maintained.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 1, 6, 11, 14, and 27 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yamazaki et al (Biol. Reprod. 59: 1439-1444, 12/1998) in view of any one of Rocamora et al (Biochem. J. 267: 821-829, 1990), Inpanbutr et al (Histochem. 97: 335-339, 1992), or Vilagrasa et al (Mol. Reprod. Dev. 47: 26-29, 1997).

Yamazaki taught a method of delivering DNA to a spermatogonium in a mouse by injecting said DNA into a mouse testicle in vivo and then applying an electrical current to the testicle to enhance uptake of the DNA by spermatogonial cells. Yamazaki indicated that the method was useful as a simple assay system to analyze regulatory elements of spermatogenic genes such as spermatogenic specific enhancer elements, and that spermatogenesis is an excellent model system to study the molecular mechanism responsible for switching from mitotic to meiotic cell division. See abstract; page 1439, column 2, second full paragraph; and page 1442, column 2, first full paragraph. Finally, Yamazaki taught that electroporation can be used for any type of tissue or cell. See first sentence of paragraph bridging pages 1442 and 1443.

Yamazaki did not teach DNA delivery in a chicken.

Rocamora studied the expression and methylation of Ubl and UblI polyubiquitin genes during maturation in chick testis. Adult and 8 week old sexually immature birds were used. The authors characterized unmethylated CpG islands in the 5' proximal noncoding region, as well as methylation in 5' proximal and distal sites. The authors noted that expression of UblI was regulated in a tissue specific manner, and indicated that further analysis of the 5' region might allow clarification of the control of expression during spermatogenesis. See entire document, especially abstract; page 82, column 1 first and second full paragraphs; page 828, column 1, fourth full paragraph, and paragraph bridging columns 1 and 2.

Inpanbutr studied expression of Calbindin-D_{28k} in growing chick testes. Calbindin presence was assayed in the embryo, and at 1 day, 1 week, 5 weeks, 9 weeks, and 12

weeks after hatching. The authors indicate that the changes in expression over this time course suggest the involvement of calbindin in spermatogenesis or cell division, and that agent(s) responsible for regulating expression of calbindin are unknown. See abstract; Figures 1-8 (legends on page 336, Figures on page 337); paragraph bridging columns 1 and 2 on page 338; and page 338, column 2, lines 5-9 of second full paragraph.

Vilagrasa examined the expression of Bcl-2 and Bcl-x in the testes of embryonic chickens, immature 5-week old chickens, and mature 20-week old chickens. See abstract; sentence bridging columns 1 and 2 on page 26; first and second full paragraphs of column 1 on page 28; Fig. on page 28; and Fig. 3 on page 29.

It is clear from the studies of Rocamora, Inpanbutr, and Vilagrasa that those of ordinary skill in the art prior to the time of the invention were interested in the patterns of expression of a variety of genes in the testes of chickens prior to and after puberty. It would have been obvious to one of ordinary skill in the art at the time of the invention to study the regulatory regions of any of these genes by using the electroporation approach of Yamazaki in chicken testes. One would have been motivated to do so because it would allow the analysis of discrete fragments of the regulatory regions in order to isolate and identify enhancers and transcription factor binding sites that have a role in differential expression during development. In so doing one would clearly be motivated to analyze transcriptional activity and or factor binding activity of these sequences at different times in development corresponding to the times studied by these various investigators both before and after puberty. One would have had a

Art Unit: 1635

reasonable expectation of success because, as indicated by Yamazaki, electroporation is highly adaptable to a variety of tissue and cell types, so one of skill would have reasonably expected to be able to successfully electroporate chicken testicles. Claim 27 is included in this rejection because production of sperm from the transfected spermatogonia containing the delivered DNA is considered to be inherent. Thus the invention as a whole was prima facie obvious.

Claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yamazaki and any one of Rocamora, Inpanbutr, or Vilagrasa as applied to claims 1, 6, 11, 14, and 27 above, and further in view of Muramatsu et al (Biochem. Biophys. Res. Comm. 233: 45-49, 1997).

The teachings of Yamazaki, Rocamora, Inpanbutr, and Vilagrasa are summarized above. Yamazaki can be combined with any or all of Rocamora, Inpanbutr, and Vilagrasa to render obvious methods of delivering to spermatogonial cell in a chicken in vivo a DNA.

The combined references did not teach the use of a lipid or phospholipid to enhance DNA transfer.

Muramatsu taught that a variety of techniques were used to transfer nucleic acids to cells in vivo, including electroporation and lipofection. See page 45, column 2, lines 5-9. Accordingly it would have been obvious to one of ordinary skill in the art at the time of the invention to substitute lipofection for electroporation in the method of Yamazaki

as modified by any or all of Rocamora, Inpanbutr, or Vilagrasa. Thus the invention as a whole was prima facie obvious.

Claim 24 is rejected under 35 U.S.C. 103(a) as being unpatentable over Yamazaki and any one of Rocamora, Inpanbutr, or Vilagrasa as applied to claims 1, 6, 11, 14, and 27 above, and further in view of Dassouli et al (J. Mol. Endocrinol. 15(2): 129-141, 1994).

The teachings of Yamazaki, Rocamora, Inpanbutr, and Vilagrasa are summarized above. Yamazaki can be combined with any or all of Rocamora, Inpanbutr, and Vilagrasa to render obvious methods of delivering to spermatogonial cell in a chicken in vivo a DNA.

The combined references did not teach the use of a lipid or phospholipid to enhance DNA transfer.

Dassouli taught the use of DEAE-dextran to facilitate the uptake of cells in vivo. See abstract. Accordingly it would have been obvious to one of ordinary skill in the art at the time of the invention to substitute DEAE-dextran transfection for electroporation in the method of Yamazaki as modified by any or all of Rocamora, Inpanbutr, or Vilagrasa. Thus the invention as a whole was prima facie obvious.

Double Patenting

The double patenting rejection is withdrawn in view of the submission and approval of a terminal disclaimer over US 6,686,199.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 571-272-0762. The examiner can normally be reached Monday through Friday between the hours of 6:00 AM and 3:30. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, J. Douglas Schultz, can be reached at (571) 272-0763. The official central fax number is 571-273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

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Richard Schnizer, Ph.D.
Primary Examiner
Art Unit 1635